SCIENTIFIC REPORT

Eccentricity and measurement variability and repeatability with the retinal thickness analyser

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Aims: To define the variability and repeatability of retinal thickness measurements using the retinal thickness analyser (RTA) and to elucidate any interaction between eccentricity (that is, position relative to the fovea) and variability and repeatability.

Methods: The sample comprised 20 normal subjects of mean age 33 years. Each subject attended for two visits. Repeated RTA scans were acquired centred on the fovea and for any one of the four possible non-foveal scan areas. The mean retinal thickness (+SD) was calculated for a series of concentric circular bands centred on fixation. A repeated measures analysis of variance (ANOVA) was used to determine any significant interaction between the variability of RTA thickness values and eccentricity.

Results: The group mean coefficient of variation and coefficient of repeatability were highest at the fovea. The repeated measures ANOVA revealed that the within test variability of RTA measurements varied significantly with eccentricity ($p<0.0001$). Similarly, the between test repeatability varied significantly with eccentricity ($p=0.045$).

Conclusion: The significantly elevated within test variability and between test repeatability in the foveal area need to be considered when using the RTA to evaluate patients with macular disease.

Ocular diseases that result in alterations of retinal thickness include diabetic macular oedema (DMO), glaucoma, age related maculopathy, vascular occlusion, and macular hole. Established clinical techniques are non-quantitative and relatively insensitive to small changes in retinal thickness because they rely on the subjective assessment of the clinician.[1] Reliable, quantitative, and sensitive methods to determine retinal thickness will lead to more accurate diagnosis and effective management. For example, the retinal thickness analyser (RTA; Talia Technology Ltd, Neve-IIan, Israel) has been extensively evaluated in clinically normal subjects,[2–8] patients with various retinal diseases,[9–27] and following various interventions.[28–31] Retinal thickness measurements using the RTA have been shown to correlate with other techniques[7 27 32] and histological assessment.[4 7] Importantly, the within session global (as opposed to local) variability of RTA derived retinal thickness measurements has been estimated to be approximately 11–23 μm, while the between session repeatability was approximately 11–31 μm.[2–5 8 10 16] Despite previous anecdotal comments about a possible effect,[2] no studies have systematically examined the relation between RTA measurement variability and repeatability and eccentricity. To the best of our knowledge, this is the first study to elucidate an interaction between eccentricity and RTA measurement variability and repeatability. For the purposes of this study, eccentricity was defined as the distance of the retinal thickness measurement (coordinates x, y) relative to the position of the fovea (coordinates 0, 0).

PATIENTS AND METHODS

Sample

The sample comprised 20 normal subjects of mean age 33 years (SD 8 years, range 22–51 years). Informed consent was obtained from each subject. The study followed the tenets of the Declaration of Helsinki and was approved by the University of Waterloo Office of Research Ethics and the Toronto University Health Network Research Ethics Board. One eye of each subject was assigned to the study: 10 right and 10 left eyes were selected. Inclusion criteria comprised a logMAR visual acuity of 0.0 or better, and a normal fundus appearance. Visual acuity was assessed using a 96% contrast Regan logMAR chart. Stereo fundus biomicroscopy, through a dilated pupil, was carried out to ensure the exclusion of significant ocular pathology. Exclusion criteria included a distance refractive error of greater than plus or minus 6.00 dioptres sphere and/or plus or minus 1.50 dioptres cylinder, a history of ocular disease, or surgery, and a family history of glaucoma or diabetes in a first degree relative. Subjects with significant lenticular opacities, as assessed by the Lens Opacity Classification System III[33] were excluded: significant lenticular opacity was defined as nuclear colour >2; nuclear opalescence >2; cortical cataract >1; and posterior subcapsular cataract >1. Recent publications have demonstrated that RTA measurement can be adversely affected by lenticular opacity.[8 27]

Retinal thickness analyser

The RTA comprises a laser slit biomicroscope and digital camera attached to an ophthalmic table, a patient headrest, and a personal computer (software version 4.075). In brief, a green helium-neon laser light of 543 nm wavelength is scanned across the retina to produce 16 discrete slit images within a 3 mm × 3 mm area of retina. The reflected slit images are recorded digitally. Retinal thickness is derived from the separation between the anterior (that is, at, or close to, the internal limiting membrane, ILM) and posterior (that is, at, or close to, the retinal pigment epithelium, RPE) reflectance interfaces[34] for 16 points along each slit using densitometry. Consequently, the derivation of retinal thickness is dependent upon the clarity of RTA slit image. Patient fixation is aided by means of an internal fixation target that can be moved. Depth resolution and depth precision are reported to be 5–10 μm and 50 μm, respectively.[3] A more detailed explanation of the RTA optical principles has been described elsewhere.[3–5 23]

Procedures

Each subject attended for two visits within a maximum 4 week period (mean interval 7 days, range 1–30 days). At both visits, the study eye of each subject was dilated using 1%

Mydriacyl. Retinal thickness was initially assessed using the five default fixation locations of the RTA (that is, centred on the fovea, superotemporal, superonasal, inferotemporal, and inferonasal). Subsequently, the fovea centred scan area, and any one of the four possible non-foveal scan areas (since RTA thickness values are not significantly different between the four meridians[5]) were each repeated six times using an alternating paradigm. The position of the non-foveal scan was constant for a given subject but was systematically varied between subjects. Only the fovea centred and the selected non-foveal scans, from the initial five default scans, were included in the analysis. A single, experienced RTA operator was used throughout (EG).

Analysis

Circular band analysis

This method of analysis was chosen since it was relatively robust to misalignment of successive RTA images. The scanned area of each image was divided into concentric circular bands using the radial analysis feature. Radii ranged from 200 μm to 3000 μm and successive circles, centred on the fixation target, were separated by 200 μm (fig 1). The mean retinal thickness value within each concentric circular band was calculated for each individual. The variability of the mean thickness values was compared during and between visits, for each concentric circular band, using the coefficient of variation (COV = SD/mean) and coefficient of repeatability (COR = 1.96 × SD of the differences between visits 1 and 2), respectively. The number of retinal thickness values used to calculate the mean varied between four (0 to 200 μm band) and 61 (1400–1600 μm band) according to the position and area of the circular band relative to the fovea.

Spoke analysis

A spoke analysis was also undertaken to negate the influence, if any, of the number of retinal thickness values used to calculate the mean in each circular band. Using the spoke analysis, the mean of three retinal thickness values in each band was calculated extending from the fovea along one of the principal meridians (that is, 45°, 135°, 225°, or 315°). The COV was then calculated for each band of the spoke as a function of visit.

Pointwise analysis

A pointwise analysis was undertaken to determine if the magnitude of retinal thickness was related to the variability of thickness measurements. Images were registered across visits (that is, aligned) and the mean and SD of thickness values of all points was calculated. The mean and SD of the pointwise thickness data in the seven scans within each session were plotted to elucidate any relation between measurement variability and retinal thickness.

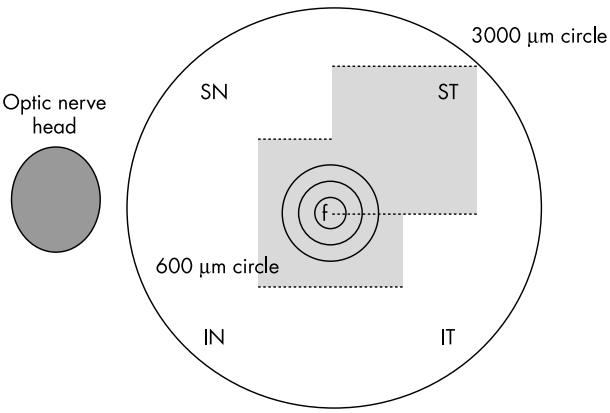

Figure 1 Schematic diagram showing the default RTA scan locations (f = fovea, SN = superonasal, ST = superotemporal, IN = inferonasal, IT = inferotemporal) and the radii used for the circular band analysis (ranging from 200–3000 μm radii, centred on the fixation target, in 200 μm steps).

Statistics

A repeated measures analysis of variance (ANOVA) was used to determine any significant interaction between the variability of RTA thickness values and eccentricity. Variability of retinal thickness was the dependent variable; visit (that is, 1 or 2) and eccentricity were the within subject factors. Intraclass correlation coefficients were also calculated to determine the reliability of the RTA thickness measurements across visits.

RESULTS

The group mean profile of retinal thickness is shown in figure 2A. The group mean retinal thickness was thinnest at the fovea (136.2 μm, SE 6.9), thickest at 1000–1800 μm from the fovea (186.7 μm, SE 4.8) and then declined with further increase in eccentricity (163.6 μm, SE 4.7). Individual retinal thickness values ranged from 86.7 μm to 219.8 μm (median value 133.5 μm) for the central radius (0–200 μm from the fovea).

The group mean COV of retinal thickness is shown in figure 2B. It was highest at the fovea (11% averaged across two visits, range of individual values 3% to 25%), reached a minimum between 600–1200 um (3.5% averaged across two visits, range of individual values 1%–8%) before increasing again with further increase in eccentricity (5% averaged across two visits, range of individual values 1%–13 %).

The group mean COR of retinal thickness is shown in figure 2C. It was highest at the fovea (40.3 μm compared with a mean effect of 136.2 μm), lowest at 600–1000 μm (16.6 μm compared with a mean effect of 172.7 μm) eccentricity, then increased up to 2000–2200 μm (25.5 μm compared with a mean effect of 175.6 μm) eccentricity and subsequently decreased with further increase in eccentricity (21.5 μm at 2600–3000 μm compared with a mean effect of 164.3 μm).

The repeated measures ANOVA revealed that the variability in retinal thickness measurements across visits was not significant ($p = 0.455$). The variability in retinal thickness measurements (that is, within visit) as a function of eccentricity was significant ($p<0.0001$). Similarly, the variability in retinal thickness measurements across visits (that is, repeatability) as a function of eccentricity was significant ($p = 0.045$). Intraclass correlation coefficients calculated for each eccentricity ranged between 0.79 (1800–2000 μm) and 0.91 (2600–2800 and 2800–3000 μm) and were significantly correlated across visits ($p<0.0001$).

The spoke analysis demonstrated a similar relation between COV and eccentricity, and similar magnitudes of COV, to that of the circular band analysis. The pointwise analysis showed that there was no relation between retinal thickness and the variability of the retinal thickness measurements.

DISCUSSION

This study describes the local variability and repeatability of retinal thickness measurements using the RTA in a group of normal subjects with minimal, if any, media opacities. It is the first study to elucidate the interaction between eccentricity and RTA measurement variability and repeatability. Within test variability was found to vary significantly with change in eccentricity from the fovea ($p<0.0001$). Similarly, the between test repeatability was found to vary significantly

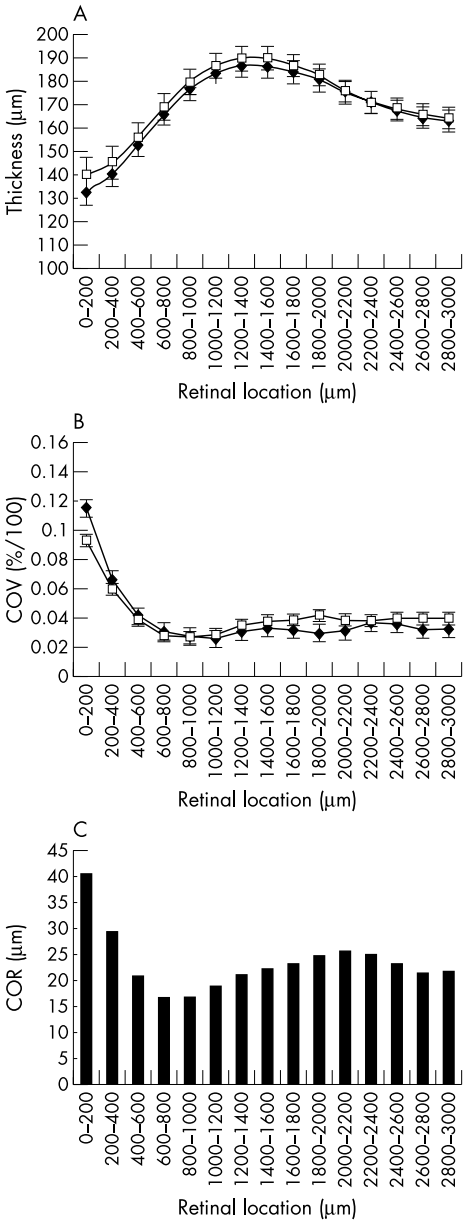

Figure 2 (A) Group mean profile of retinal thickness (0–3000 μm) derived using the circular band analysis for visits 1 (solid circle) and 2 (open square). (B) Group mean profile of COV derived using the circular band analysis for visits 1 (solid circle) and 2 (open square). (C) Bar chart showing group mean COR derived using the circular band analysis. For graphs A and B, the error bars represent plus or minus 1 standard error of the mean (COV = coefficient of variation. COR = coefficient of repeatability).

with eccentricity ($p = 0.045$). The within test variability and between test repeatability were both most pronounced at the fovea.

The retinal thickness values obtained from our sample are generally in good agreement with previous histological studies.[35 36] Hogan and coworkers[35] reported mean foveal thickness values of 130 μm and while Straatsma and coworkers[36] reported values for foveal thickness of 100 μm. Conversely, Fine and Yanoff[37] reported mean foveal thickness values of 200 μm based upon histological techniques. Generally, with the exception of Konno and coworkers,[7] published RTA studies have tended to find higher retinal thickness values when compared with our retinal thickness values. The difference in the magnitude of retinal thickness between the results reported in this and previous RTA studies may be attributed to the strict exclusion criteria employed in this study in relation to lens opacity. The light scattering effect induced by media opacities will result in blurred retinal slit images and an artefactual increase of retinal thickness values. Interestingly, a more recent study has found values of mean retinal thickness that are in very close agreement with our findings.[8]

The variability and repeatability of RTA measurements have been reported in various published studies.[2–5 8 10 16] We have found global mean COV values ranging from 10.6 μm to 23 μm. The retinal thickness values of any individual need to exceed the normal range of values to an extent greater than the magnitude of the COV before a significant difference in retinal thickness can be claimed. However, these studies have assessed variability over relatively large scan areas and did not address the issue of the interaction of RTA measurement variability with eccentricity. The magnitudes of COV found in this study are similar to those previously reported when relatively large scan areas are compared (by interpolation of areas under the COV profiles in fig 2B).

Change in retinal thickness over time of any individual needs to exceed the magnitude of the COR before significance can be claimed. Previous studies[2–4 10 16] evaluated repeatability by calculating the standard deviation of RTA measurements divided by the mean (across two visits). These studies found repeatability values ranging from 10.8 μm to 19 μm but did not consider any possible interaction of RTA repeatability and eccentricity and therefore calculated average indices that reflected repeatability for relatively large scan areas. The magnitudes of COR found in this study are high compared with those previously reported. This can be explained in part by the use of the COR index (which represents 1.96 × SD of the differences in RTA measurements across visits) and, more importantly, by the local variation in COR revealed in this study. In addition, the intraclass correlation coefficients demonstrated that the data were highly correlated within and between visits.

The circular band analysis resulted in a different number of data points within each circular band area—that is, 28 (4 points × 7 scans) to 427 (61 points × 7 scans). The spoke analysis (which resulted in three data points within each circular band) demonstrated a similar relation between COV and eccentricity, and similar magnitudes of COV, with that of the circular band analysis. We conclude that the variation in COV as a function of eccentricity is not attributable to the differences in sampling rate across the circular bands.

The pointwise analysis demonstrated no relation between retinal thickness and the variability of the retinal thickness measurements (as assessed by the SD of the seven scans). The COV and COR are most prominent in areas of greatest change in retinal topography, particularly in the region of the foveal pit. This may in part be explained by the possible impact of involuntary physiological eye movements and reduced nerve fibre layer (NFL) thickness at the fovea resulting in a localised reduction of reflectance intensity. Also, the ability of the RTA software algorithm to identify the anterior and posterior reflecting slit interfaces may be limited in areas of minimal retinal thickness. The possible impact of involuntary physiological eye movements as a further contributor to this local variation in RTA measurement variability and repeatability cannot be excluded. Interestingly, the test-retest measurement variability of confocal scanning laser tomography has also been demonstrated to be significantly greater in areas of greatest change of retinal topography.[38 39] Such an effect can be anticipated to impact upon other reflectance based scanning laser imaging systems.

We report a significant interaction between eccentricity and RTA measurement variability and repeatability. The group mean COV (11% at fovea and 3.5%–5.0% elsewhere) and COR (40.3 μm at the fovea and 16.6–25.5 μm elsewhere) were highest at the fovea. Measurement variability and repeatability was not attributable to the magnitude of retinal thickness but, instead, was most evident in areas of greatest change of retinal topography. The exaggerated within test variability and between test repeatability in the foveal area needs to be considered when using the RTA to evaluate patients with macular pathology. Confidence limits to determine abnormality relative to a normal database, and change in retinal thickness relative to baseline for a given individual, need to take into account the increased variability and repeatability in areas of greatest rate of change in retinal topography in order to maximise the sensitivity of the RTA.

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